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(54) Monoclonal antibodies reactive with shared idiotypes on human antibodies to native DNA from patients with systemic Lupus erythematosus.

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Description

Background of the invention

The pres nt invention relates to a monoclonal antibody capable of reacting with an idiotype on human anti-native DNA antibodies shared between two or more genetically nonidentical individuals of the human population. The invention also relates to diagnostic test methods to detect the presence of anti-native DNA antibodies in patients suspected of having systemic lupus erythematosus.

The invention relates also to a test for determining a anti-native DNA antibody.

The term "antibody" as used in immunology is a generic term and is used to cover numerous immunoglobulin molecules which may be alike in some respects and different in other respects. Antibodies sharing a common effector function are referred to as isotypes and include the major immunoglobulin classes IgA, IgD, IgE, IgG and IgM, each of which is constructed from a heavy polypeptide chain characteristic of the particular class and a light polypeptide chain (either kappa or lambda), the heavy and light chains being joined by disulfide bridges. Heterologous antibodies, i.e. those raised in one species against the antibodies of another species, are useful in recognizing isotypes.

Antibodies of a given isotype, e.g., IgG, can be subdivided further on the basis of the particular type of heavy chain from which they are composed, e.g. $\gamma 1$, $\gamma 2$, $\gamma 3$, etc. Antibodies having the same general type of heavy chain can still show some heterogeneity in the heavy chain. Within a given animal species, certain regions on the heavy chain of a given isotype may be the same or different. Those antibodies showing similarity within these regions are referred to as allotypes. The similarity is believed to be of genetic origin.

Finally, antibodies of a given isotype and allotype may differ on the basis of their structure in or near their antigen binding regions (Fab region). Antibodies showing similarity in this region are said to be of the same idiotype. Particular discrete antigenic sites within these regions are referred to as idiotypic determinants or frequently just idiotypes. Antibodies having common or shared idiotypes generally have the same antigenic specificity.

Despite common antigenic specificity among idiotypes, not all idiotypic determinants are located in the antigen binding site of an immunoglobulin molecule. This has been demonstrated by the fact that a second antibody directed against an idiotypic determinant on the first antibody (the second antibody being referred to hereinafter as an anti-idiotypic antibody) and the antigen do not always compete for the antigen binding site on the first antibody. Idiotypic determinants are controlled by both genetic and antigenic influences. Antibodies from genetically different individuals which share a common antigenic specificity usually exhibit idiotypic heterogeneity. That is, even though the antibodies are capable of binding the same antigen, the structure of idiotypic determinants, even when located in the antigen binding site, are different.

Antibodies directed against idiotypic determinants on immunoglobulin molecules provide unique probes for detecting antibodies associated with and characteristic of auto-immune diseases. Serum containing anti-idiotypic antibodies has been used to demonstrate shared idiotypes on immunoglobulins from unrelated patients in a number of auto-immune diseases including myasthenia gravis, Graves Disease, cold agglutinin disease, and rheumatoid arthritis. Antibodies to native DNA (anti-nDNA) produced by inbred mice with diseases resembling human systemic lupus erythematosus (SLE) have been shown to share a common idiotype (Rauch et al., Journal of Immunology 129, No. 1, 1982, pages 236—241). However shared idiotypy has not previously been demonstrated in the anti-nDNA antibodies of human patients with SLE.

Systemic lupus erythematosus is a disease which is being diagnosed with ever-increasing frequency. A minimal estimate of its incidence is 10 per 100,000 people, but it occurs preferentially in blacks and in women of child-bearing age. Its incidence in family members of patients with SLE is at least one hundred fold greater than its incidence in the population at large, but the genetics of inheritance remain unclear. Systemic lupus erythematosus is a disease of protean manifestations both clinically and serologically. Clinically, SLE can affect any organ system. It causes constitutional systems such as fever and malaise. It causes arthritis, serositis (plural, peritoneal, and pericardial), renal disease, hematologic abnormalities, and central nervous system disturbances. The sera from lupus patients are capable of reacting with a variety of antigens due to the presence of auto-antibodies. These antibodies are directed primarily against nuclear antigens and cell membrane antigens, although some antibodies to cytoplasmic antigens have been described. The auto antibody which is specific for SLE and most commonly found in the serum from SLE patients is antibody to native DNA.

As stated earlier, one individual generally produces many different antibodies against a given antigen such as DNA. While all such antibodies are capable of reacting with the common antigen, the antibodies may xhibit structural differences, thereby imparting idiotypic heterogeneity. Furthermore, additional idiotypic difference will generally exist between antibodies produced by genetically nonidentical individuals against a c mmon antigen. Consequently, one w uld expect that in the genetically diverse human population, there would be wide heterogeneity in anti-DNA antibodies from patients with SLE. Therefore, an anti-idiotypic antibody produced against an anti-DNA antibody fr m one individual would not be expected to cross react with anti-DNA antibodies fr m other genetically nonidentical individuals within the population. In the present inventi n, mon clonal anti-idi typic antibodies have been produced against the anti-native DNA antibodies from one SLE pati nt which, unexpect dly, do cross react with anti-

nDNA antibodies from genetically nonidentical SLE patients within the human population. This unexpected cross-reactivity allows these monoclonal antibodies to be used as diagnostic reagents to detect anti-nDNA antibody in serum from suspected SLE patients within the population at large.

Disclosure of the Invention

In one aspect, the present invention comprises a monoclonal antibody capable of reacting with an idiotype on human anti-native DNA antibody shared between two or more genetically non-identical individuals of the human population. The inventive antibody is also referred to herein as monoclonal, anti-idiotypic antibody.

In another aspect, the present invention is a method for detecting anti-native DNA suspected of being present in a liquid biological sample, comprising contacting the sample with monoclonal, anti-idiotypic antibodies capable of binding to shared idiotypes on human anti-native DNA antibody, and determining the extent of binding.

Detailed description of the invention

The anti-idiotypic antibody of the present invention is monoclonal (derived from a single hybridoma) and is of either the IgG or IgM isotype, the former being preferred. Additionally, the antibody is not reactive with nonidiotypic regions of normal human immunoglobulin. The antibody can bind to an idiotypic determinant located in the antigen binding region of the anti-nDNA antibody or to an idiotypic determinant located outside the antigen binding region, the latter being preferred because of the ability of the monoclonal antibody to detect circulating immune complexes. The antibody cross reacts with idiotypes shared between two or more genetically nonidentical individuals.

The monoclonal, anti-idiotypic antibodies of this invention can be made using known cell fusion techniques. See, for example, Gefter et al., Somatic Cell Genet., Volume 3, pp. 231—236 (1977) and Kearney et al., J. Immunol., Volume 123, pp. 1548—1550 (1979). In general, a B lymphocyte from a mammalian organism (human or nonhuman) which has been specifically sensitized to anti-native DNA antibodies from an organism of either the same or different species as that from which the lymphocyte is obtained is fused with a compatible fusion partner which is preferably a nonsecreting myeloma cell. The resulting fusion products are then screened for those hybridomas which secrete antibody which meets the criteria set forth above.

The selected hybridomas can then be expanded by known techniques including passage as ascites.

In a preferred embodiment, the specifically sensitized B cell is made as follows. Serum from an SLE patient is reacted with DNA, generally from an extraneous source such as calf thymus. An immune complex of DNA and anti-DNA is allowed to form. The complex is isolated using, for example, ion exchange chromatography, and dissociated, generally by treatment with 8M urea. The anti-DNA antibody is then recovered using, for example, DEAE-cellulose chromatography. An alternate method for obtaining purified human anti-nDNA antibody comprises the generation of a human×human hybridoma which secretes anti-nDNA antibody. A preferred method for generating such a hybridoma is the fusion of peripheral blood lymphocytes from an SLE patient whose serum contains high levels of anti-DNA antibody with a drug marked human myeloma cell. After fusion, the hybridoma supernatant fluid can be screened for anti-DNA antibody using conventional assays such as the Millipore filter assay described below. The recovered anti-DNA antibody produced by either of these methods is then used as an immunogen to sensitize B lymphocytes in an immunocompetent recipient animal, generally a mouse. A preferred strain of mouse is BALB/c. After immunization, the mouse is sacrificed and splenocytes recovered for fusion with appropriate fusion partners. A preferred fusion partner for a sensitized mouse splenocyte is the non-producing, thioguanine-resistant myeloma line X63Ag8.653 which is on deposit at the American Type Culture Collection (Rockville, Maryland, U.S.A.) and catalogued as ATCC CRL-1580. Following fusion of lymphocyte and fusion partner to produce hybridomas, hybridoma supernatant fluids are screened for the presence of antibody capable of reacting with anti-DNA antibody. A preferred method of screening is a sandwich solid phase radioimmunoassay in which hybridoma supernatant fluid is contacted with a solid phase having coated thereon anti-DNA antibody (normal human immunoglobulin is used as a control). Any antibody in the supernatant fluid capable of binding to antigenic determinants on the anti-DNA antibody will be adsorbed onto the solid phase. Finally, a labelled antibody directed against nonidiotypic determinants on the hybridoma produced antibody is contacted with the solid phase and the extent of immunospecific binding determined by means of the label which can be an enzyme, radioisotope, chromophore or fluorophore. To distinguish hybridoma supernatant fluids containing antibody directed against isotypic and allotypic determinants on the anti-DNA antibody from supernatant fluids containing antibody directed against idiotypic determinants, the amount f immunospecifically adsorbed label on s lid phase pre-coated with anti-DNA antibody is c mpared to the am unt of immunospecifically adsorbed label on solid phase pr -coated with normal human immunoglobulin (control). Hybridoma supernatant fluids accounting for greater immunospecific label adsorption on solid phase pre-c ated with anti-DNA antibody than s lid phase pre-coat d with normal human immunogl bulin are c nsidered t contain anti-idiotypic antibody. Those supernatant fluids which acc unt for approximately equal or greater label adsorption on solid phase pre-c ated with normal human immunoglobulin as compared to solid phase pre-coated with anti-DNA antibody are consider d to contain anti-isotypic and/or anti-allotypic antibody.

To determine whether the anti-idiotypic antibody is capable of reacting with shared idiotypes, the screening described above can be repeated using anti-DNA antibody from at least two genetically nonidentical individuals. Hybridoma supernatant fluids showing reactivity with at least two anti-DNA antibodies are considered as capable of reacting with a shared idiotype on human anti-native DNA antibody and, therefore, within the scope of the present invention.

Because of their ability to detect circulating immune complexes of DNA and anti-native DNA in patient serum, the most preferred antibody of the present invention is one which is capable of reacting with a shared idiotypic determinant on human anti-native DNA antibody which is located outside the DNA binding region. Hybridomas secreting this most preferred antibody can be identified by reacting anti-DNA antibody and hybridoma supernatant fluid pre-screened for anti-idiotype activity in the presence of a large excess of free DNA. If the binding of the monoclonal antibody in the supernatant fluid and the anti-DNA antibody is not inhibited, the monoclonal antibody may be directed against an idiotypic determinant on anti-DNA antibody located outside of the DNA binding site. To further demonstrate that the putative monoclonal anti-idiotypic antibody is not binding to DNA, itself, a coprecipitation assay can be performed. In this assay, radiolabelled, generally Iodine-125 labelled, DNA is incubated with anti-DNA antibody or a negative control, the former preferably from an SLE patient with high titer anti-nDNA activity and the latter from a non-SLE control patient. The amounts of radiolabelled DNA and anti-nDNA antibody are chosen to avoid precipitate formation. Next supernatant fluid pre-screened for anti-idiotypic activity or a control, preferably supernatant fluid from a mouse myeloma cell line secreting a monoclonal antibody of the same isotype as the anti-idiotypic antibody, but which is non-reactive with human immunoglobulin, is added. Finally, a heterologous antiserum capable of reacting with both the monoclonal anti-idiotypic antibody and the antibody control is added in an amount capable of forming an immune precipitate. The precipitate is washed and the amount of radioactivity is determined. If the amount of radioactivity in the precipitate containing the putative monoclonal anti-idiotypic antibody and the anti-DNA antibody is greater than that of the controls, it can be concluded that the monoclonal antibody does not bind DNA directly.

After the identification and selection of hybridomas which secrete monoclonal antibodies capable of reacting with shared idiotypes on human anti-native DNA antibody, the selected hybridomas can be cloned. A preferred method of cloning comprises depositing the selected hybridoma cells on agarose on a feeder layer of rat embryo fibroblasts, growing the cells to mass culture, retesting for anti-idiotypic activity, and, finally, injecting recipient animals peritoneally and tapping ascites. A preferred animal is a pristane-primed BALB/c mouse.

It is hypothesized that SLE patients secreting high levels of anti-DNA antibody will also produce auto-antibodies directed against their own anti-DNA antibodies. It is predicted that such auto-antibodies would reduce the levels of circulating anti-DNA in the serum of such patients. It would be possible to produce a human, monoclonal antibody capable of reacting with a shared idiotype on human anti-nDNA antibody by direct fusion of peripheral blood lymphocytes from an SLE patient in serological remission (namely, when levels of circulating anti-nDNA antibody are low) with a drug marked human myeloma cell. The supernatant fluid of a hybridoma so produced would be screened for human, monoclonal anti-idiotypic antibody by the following screening procedure. A solid phase, generally the well of a microtiter plate is coated with purified Fab fragments of human anti-DNA antibody. Solid phase coated with purified Fab fragments of normal human immunoglobulin is used as a control. Hybridoma supernatant fluid is contacted with the solid phases and unadsorbed fluid washed away. Finally, the solid phases are contacted with a labelled heterologous antibody specific for the Fc region of human immunoglobulin. Greater binding of label to the solid phase coated with Fab fragments of human anti-DNA as compared to the control is evidence of the presence of monoclonal anti-idiotypic antibody. To determine whether the anti-idiotypic antibody is capable of reacting with shared idiotypes, the screening procedure is repeated using anti-DNA antibody from at least two genetically nonidentical individuals. Hybridoma supernatant fluids showing reactivity with at least two anti-DNA antibodies are considered as capable of reacting with a shared idiotype on human anti-native DNA antibody, and, therefore, within the scope of the present invention.

The antibody of the present invention can be used to detect the presence of anti-nDNA antibody in biological fluids by a variety of techniques which are well known in the art. In all of these techniques, the monoclonal, anti-idiotypic antibody of this invention is contacted with a sample suspected of containing anti-nDNA and the amount of binding determined. Among the suitable techniques are radioimmunoassay (RIA, solid or liquid phase), enzyme-linked immunosorbent assay, heterogeneous immunoassay (both competitive and noncompetitive) using labels other than enzymes and radioisotopes, homogeneous immunoassays based on fluorescence quenching and enzyme channelling, immune precipitation (including radial immune diffusion) and agglutination assays based on visual semi-quantitative detection or quantitative turbidimetric d tection. In one preferred m de, the monoclonal, anti-idiotypic antibody is used in a liquid phase RIA in which a labelled monoclonal, anti-idiotypic antibody is r acted with a sample whereby immune complex forms. The label can be a radiois tope such as Iodine-125 or an nzyme such as horseradish peroxidase, α-galactosidase or alkaline phosphatase. After a suitable reaction time, a reagent capable of precipitating the immun complex is added. A preferred reagent is a heterologous antibody reactive with the anti-nDNA antib dy. The am unt of label in th precipitate is determined and compared to positive and negative controls. In a third aspect, the present invention is a kit for determining the amount of human anti-native DNA antibody suspected of being present in a liquid sample comprising:

(i) anti-native DNA antibody positive and negative controls;

(ii) a labelled monoclonal, anti-idiotypic antibody capable of reacting with an idiotype on human anti-native DNA antibody shared between two or more gen tically nonid ntical individuals of the human population;

(iii) reagent capable of precipitating immune complexes of anti-nDNA antibody and said monoclonal anti-idiotypic antibody; and

(iv) directions for using the kit.

In another preferred mode, the test sample is coated on a solid phase such as the interior wall of a polystyrene test tube or microtiter plate well. After washing away unadsorbed sample, the solid phase is contacted with the monoclonal, anti-idiotypic antibody of this invention. After a suitable incubation period, unreacted monoclonal antibody is washed away. Finally, the solid phase is contacted with an enzymatically labelled or radiolabelled heterologous antibody reactive with the monoclonal antibody. After a suitable incubation period, the unreacted labelled antibody is washed away, and the amount of immunospecifically adsorbed label measured. If the monoclonal antibody is of mouse origin, a preferred radiolabelled heterologous antibody is Sulfur-35-methionine labelled rat anti-mouse light chain antibody.

Example

Ten ml of serum from an SLE patient (A.W.) containing anti-DNA antibodies was mixed with 10 ml of 0.01M Na phosphate buffer, pH 6.6, containing calf thymus DNA (Type 1, Sigma Chemical Co., St. Louis, MO) at a concentration of 0.5 mg/ml. This mixture was incubated for 1 hour at room temperature followed by 16 h at 4°C to allow DNA/anti-DNA antibody complexes to form and was then applied to a 300 ml DE-52 column (Whatman Ltd., Clifton, N.J.) equilibrated with 0.01M Na phosphate (pH 6.6) containing 0.1M NaCl. The complexes were eluted with 0.5M NaCl, dissociated with 8M urea and rechromatographed on a DE-52 column to yield an enriched preparation of human anti-DNA antibodies of the IgG class which were free of native DNA.

Five BALB/c mice were immunized by intraperitoneal injection with 100 µg of an immunogen which is the enriched anti-DNA antibody emulsified in complete Freund's adjuvant. The mice were boosted weekly for 6 to 10 weeks with the immunogen without adjuvant. Three of the five mice were shown, using a solid phase radioimmunoassay (see below), to have produced high levels of antibodies against the immunogen. Spleen cells from these three immunized mice were fused to thioguanin-eresistant non immunoglobulin secreting, mouse myeloma cells (line X63Ag8.653) and plated in 96 well microtiter plates. Supernatant fluids from those cultures containing viable hybridomas were screened to detect clones producing antibodies reactive with purified immunogen using a solid phase radioimmunoassay. Samples of each culture supernatant fluid were incubated in wells of Immulon-II (Dynatech Labs., Inc., Alexandria, VA) microtiter plates which had been precoated by overnight incubation with either purified anti-DNA antibodies from an SLE patient or with purified normnal human immunoglobulin. After extensive washing of the coated plates, bound mouse immunoglobulin was detected by addition of a Sulfur-35-methionine labelled monoclonal rat anti-mouse kappa light chain antibody. Differential binding of radiolabel to the anti-DNA plate relative to the normal human immunoglobulin plate was taken as evidence for the presence of a putative anti-idiotypic antibody specific for anti-DNA in the culture supernatant fluid of a given hybridoma. Five of such hybridomas were identified as shown in the Table.

Identification of hybridomas producing anti-idiotype

	Anti-DNA antibodies cpm	Control human s rum, cpm
Positive control*	15,226	8,554
	18,195	11,402
Control**	135	120
	112	109
Hybridoma		
3I	2,107	157
	1,885	135
9F	2,255	154
	1,942	114
17A	306	131
	332	211
3H	223	124
	192	102
9E	152	100
	160	99

Either enriched AW anti-DNA antibodies or control serum was absorbed to polystyrene wells. Mouse serum or hybridomas supernatant was added, followed by radiolabelled rat anti-mouse light chain. Duplicate values are shown.

* Serum from a mouse that had been immunized at a dilution of 1:100.
** Supernatant from an irrelevant hybridoma line.

Cells from these hybridomas were further cloned in agarose on a feeder layer of rat embryo fibroblasts. Clones were picked, grown to mass culture and retested for anti-idiotypic activity. Pristane-primed BALB/c mice were injected intraperitoneally with 1×10^7 hybridoma cells, and the ascites fluid was harvested 10—14 days later.

The monoclonal antibody hereinafter designated 3I which was produced by one of these clones was further characterized as follows. Ouchterlony analysis indicated 3I to be a mouse IgG_1 immunoglobulin with kappa light chains. Activity of 3I against anti-DNA antibodies was not inhibited by a fifty-fold excess of normal human serum. Furthermore, its binding to anti-DNA antibody was not blocked by the addition of a large excess of free DNA. 3I did not contain anti-DNA activity as measured in a conventional Millipore filter assay. (Iodine-125 labelled DNA is reacted with a dilution of serum suspected of containing anti-DNA antibody. Immune complex is trapped on a .45 µm filter which is then counted for radioactivity). Furthermore, 3I antibody when combined with a rabbit anti-mouse antibody was capable of precipitating radiolabelled DNA only if the DNA had been preincubated with anti-DNA antibodies from patient sera. Finally, 3I was found to bind to the Fab region of anti-DNA antibodies.

From these data, It was concluded that the 3I antibody was anti-idiotypic and was specific for an idiotype present on a species of anti-DNA antibodies from patient AW. Furthermore, the 3I antibody is not directed against the antigen combining site of anti-DNA antibody because binding to anti-DNA is not blocked by the addition of excess DNA, and because it binds to DNA/anti-DNA antibody complexes.

Sera obtained from active SLE patients and normal healthy donors were tested to determine their reactivity with 3I using a solid phase radioimmunoassay. In this assay, the sera, diluted 1/10 in phosphate buffered saline (PBS), were incubated overnight at 4°C in the wells of an Immulon-II microtiter plate. Plates were washed three times with PBS and then incubated for 1 hour with PBS containing 5% (w/v) bovine serum albumin (BSA). Culture supernatant fluid or a 1/50 dilution of ascites containing 3I was added to each well. After 90 minutes incubation at room temperature, the plates were washed with a washing s lution containing 0.5% Tween® 20, 0.15M NaCl and 2% BSA (pH 8.3). Sulfur-35-methionine-labelled monoclonal rat anti-mouse kappa light chain antibody (clone 187.1, ATCC-HB 58) was added and the plates further incubated at room temperature f r 90 minutes. After xtensive washing with the washing solution, the extent of binding was det rmined in each well by scintillation counting.

Eight of nine active SLE patients pr viously shown to have high levels of anti-DNA antibodies show d significant reactivity with 3I. Furthermore, four of nine active patients without significant reactivity in the

Millipore filter assay for anti-DNA antibodies also reacted with 3I. Many of these sera from patients with SLE reacted with 3I more strongly than did serum from patient AW, from which the anti-DNA antibody was purified. In contrast, none of the ten normal donors showed significant 3I-reactivity in their sera.

Serum samples were obtained from thirteen patients during active disease and during clinical remission. Remission sera from all patients showed a loss of anti-nDNA antibody in the Millipore filter assay. Six of these remission sera showed a concomitant loss of 3I reactivity in the solid phase radioimmunoassay. However, the remaining seven remission sera showed little or no change in 3I reactivity. The continued reactivity of these seven sera with 3I suggests that anti-nDNA antibodies were present, but were prevented from reacting with DNA in the Millipore filter assay. Therefore an assay for detecting anti-nDNA in serum samples using 3I as a reagent has greater diagnostic and prognostic utility than anti-nDNA assays of the prior art. The hybridoma secreting 3I has been deposited in accordance with the provisions of the Budapest Treaty at the American Type Culture Collection, Rockville, Maryland, where it is catalogued as ATCC HB 8376.

Claims for the Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A monoclonal antibody capable of reacting with an idiotype on human anti-native DNA antibody shared between two or more genetically nonidentical individuals of the human population.

2. The monoclonal antibody of claim 1 which is capable of reacting with an idiotype on human anti-native DNA which is located outside the DNA binding site of the anti-native DNA antibody.

3. The monoclonal antibody of claim 1 which is of mouse origin.

4. The monoclonal antibody of claim 3 which is secreted by the hybridoma deposited at the American Type Culture Collection and catalogued as ATCC #HB8376.

5. The monoclonal antibody of claim 1 which has been produced by the following process:

(1) obtaining lymphocytes specifically sensitized to human anti-native DNA antibody;

(2) fusing the lymphocytes with fusion partners to form hybridomas;

(3) selecting those hybridomas which secrete an antibody capable of reacting with an idiotypic determinant on human anti-native DNA antibody from at least two genetically nonidentical individuals of the human population;

(4) optionally cloning the selected hybridomas; and

(5) recovering the monoclonal antibodies capable of reacting with a idiotype on human anti-native DNA antibody shared between two or more genetically nonidentical individuals of the human population.

6. The monoclonal antibody of claim 5 wherein step (1) comprises immunizing an animal with human anti-DNA antibody and isolating spleen cells from the animal.

7. The monoclonal antibody of claim 6 wherein the animal is a mouse.

8. The monoclonal antibody of claim 6 wherein the human anti-native DNA antibody is obtained from the dissociation of an immune complex comprising DNA and human anti-DNA antibody.

9. The monoclonal antibody of claim 8 wherein the human anti-DNA antibody is derived from a patient with systemic lupus erythematosus.

10. The monoclonal antibody of claim 5 wherein the fusion partner is a myeloma cell.

11. The monoclonal antibody of claim 10 wherein the meyloma cell is X63Ag8.653 which is deposited at the American Type Culture Collection and catalogued as CRL-1580.

12. A method for detecting human anti-native DNA antibody suspected of being present in a liquid sample comprising contacting the sample with a monoclonal, anti-idiotypic antibody of claim 1 and determining the extent of binding.

13. The method of claim 12 which comprises:

(i) forming a reaction mixture by contacting the sample with a labelled monoclonal, anti-idiotypic antibody of claim 1;

(ii) allowing an immunochemical reaction to take place whereby a fraction of the labelled antibody forms an immune complex with the anti-native DNA antibody and a fraction remains free;

(iii) separating the immune complex from the free labelled antibody;

(iv) measuring the label in one of the fractions; and

(v) relating the amount of measured label to the amount of human anti-native DNA initially present in the sample.

14. The method of claim 13 wherein the separation step (iii) comprises the addition to the reaction mixture of a reagent capable of precipitating the immune complex.

15. The method of claim 14 wherein the precipitating reagent is a heterologous antibody capable of reacting with the anti-native DNA antibody.

16. The method of claim 13 wherein the label is a radi isot pe, enzym , chromophore r fluorophore.

17. The meth d of claim 16 wherein the radioisotope is Iodine-125.

18. The method of claim 16 wherein th enzyme is horseradish per xidase, α-galactosidase or alkaline phosphatase.

19. The method of claim 12 which comprises:

(i) contacting the sampl with a solid phase onto which the suspected anti-native DNA antibody is adsorbed;

(ii) contacting th solid phase with a monoclonal, anti-idiotypic antib dy of claim 1 whereby an immune complex f rms on th solid phase;

(iii) contacting the solid phas with a labell d antibody capable of reacting with the monoclonal antibody of the immune complex;

(iv) measuring the amount of label adsorbed onto the solid phase; and

(v) relating the amount of label to the amount of anti-native DNA initially present in the sample.

20. The method of claim 19 wherein the solid phase is the well of a microtiter plate.

21. The method of claim 19 wherein the labelled antibody is a labelled, rat anti-(mouse light chain) antibody.

22. The method of claim 21 wherein the label is a radioisotope, enzyme, chromophore or fluorophore.

23. The method of claim 22 wherein the label is sulfur-35.

24. The method of claim 22 wherein the enzyme is horseradish peroxidase, α-galactosidase or alkaline phosphatase.

25. A kit for determining the amount of human anti-native DNA antibody suspected of being present in a liquid sample, comprising:

(i) anti-native DNA antibody positive and negative controls;

(ii) labelled, monoclonal anti-idiotypic antibody of claim 1;

(iii) reagent capable of precipitating immune complex of the anti-native DNA antibody and the labelled monoclonal, anti-idiotypic antibody; and

(iv) directions for using the kit.

26. The kit of claim 25 wherein the label is a radioisotope, enzyme, chromophore or fluorophore.

27. The kit of claim 26 wherein the radioisotope is Iodine-125.

28. The kit of claim 26 wherein the enzyme is horseradish peroxidase, α-galactosidase or alkaline phosphatase.

29. The kit of claim 25 wherein the reagent is a heterologous antibody capable of reacting with the anti-native DNA antibody.

30. The hybridoma which is on deposit at the American Type Culture Collection and catalogued as ATCC #HB8376 and mutations thereof.

Claims for the Contracting State: AT

1. Process for the production of a monoclonal antibody capable of reacting with a idiotype on human anti-native DNA antibody, shared between two or more genetically nonidentical individuals of the human population comprising:

(1) obtaining lymphocytes specifically sensitized to human anti-native DNA antibody;

(2) fusing the lymphocytes with fusion partners to form hybridomas;

(3) selecting those hybridomas which secrete an antibody capable of reacting with an idiotypic determinant on human anti-native DNA antibody from at least two genetically nonidentical individuals of the human population;

(4) optionally cloning the selected hybridomas; and

(5) recovering the monoclonal antibodies.

2. Process of claim 1 wherein step (1) comprises immunizing an animal with human anti-DNA antibody and isolating spleen cells from the animal.

3. Process of claim 2 wherein the animal is a mouse.

4. Process of claim 2 wherein the human anti-native DNA antibody is obtained from the dissociation of an immune complex comprising DNA and human anti-DNA antibody.

5. Process of claim 4 wherein the human anti-DNA antibody is derived from a patient with systemic lupus erythematosus.

6. Process of claim 1 wherein the fusion partner is a myeloma cell.

7. Process of claim 6 wherein the myeloma cell is X63Ag8.653 which is deposited at the American Type Culture Collection and catalogued as CRL-1580.

8. A method for detecting human anti-native DNA antibody suspected of being present in a liquid sample comprising contacting the sample with a monoclonal, anti-idiotypic antibody of claim 1 and determining the extent of binding.

9. The method of claim 8 which comprises:

(i) forming a reaction mixture by contacting the sample with a labelled monoclonal, anti-idiotypic antibody prepared according to claim 1:

(ii) allowing an immunochemical reaction to take place whereby a fraction of the labelled antibody forms an immune complex wjith the anti-native DNA antibody and a fraction remains free:

(iii) separating the immune complex from the free labelled antibody;

(iv) measuring the label in one of the fractions; and

(v) relating the amount of measured label to the am unt of human anti-native DNA initially present in the sample.

10. The method of claim 9 wherein the separation step (iii) comprises th addition to the r action mixtur of a reagent capable of precipitating th immune c mplex.

11. The method of claim 10 wherein the precipitating reagent is a heterologous antibody capable f reacting with the anti-native DNA antibody.

12. The method of claim 9 wherein the label is a radioisotope, enzyme, chromophore or fluor phore.

13. Th method of claim 12 wherein the radioisotope is Iodine-125.

14. The method of claim 12 wherein the enzyme is horseradish peroxidase, α-galactosidase or alkaline phosphatase.

15. The method of claim 8 which comprises:

(i) contacting the sample with a solid phase onto which the suspected anti-native DNA antibody is adsorbed;

(ii) contacting the solid phase with a monoclonal, anti-idiotypic antibody prepared according to claim 1 whereby an immune complex forms on the solid phase;

(iii) contacting the solid phase with a labelled antibody capable of reacting with the monoclonal antibody of the immune complex;

(iv) measuring the amount of label adsorbed onto the solid phase; and

(v) relating the amount of label to the amount of anti-native DNA initially present in the sample.

16. The method of claim 15 wherein the solid phase is the well of a microtiter plate.

17. The method of claim 15 wherein the labelled antibody is a labelled, rat anti-(mouse light chain) antibody.

18. The method of claim 17 wherein the label is a radioisotope, enzyme, chromophore or fluorophore.

19. The method of claim 18 wherein the label is sulfur-35.

20. The method of claim 18 wherein the enzyme is horseradish peroxidase, α-galactosidase or alkaline phosphatase.

21. A kit for determining the amount of human anti-native DNA antibody suspected of being present in a liquid sample, comprising:

(i) anti-native DNA antibody positive and negative controls;

(ii) labelled, monoclonal anti-idiotypic antibody prepared according to claim 1;

(iii) reagent capable of precipitating immune complex of the anti-native DNA antibody and the labelled monoclonal, anti-idiotypic antibody; and

(iv) directions for using the kit.

22. The kit of claim 21 wherein the label is a radioisotope, enzyme, chromophore or fluorophore.

23. The kit of claim 22 wherein the radioisotope is Iodine-125.

24. The kit of claim 22 wherein the enzyme is horseradish peroxidase, α-galactosidase or alkaline phosphatase.

25. The kit of claim 21 wherein the reagent is a heterologous antibody capable of reacting with the anti-native DNA antibody.

26. Process for the production of the hybridoma which is on deposit at the American Type Culture Collection and catalogued as ATCC #HB8376 and mutations thereof, comprising

(1) obtaining lymphocytes specifically sensitized to human anti-native DNA antibody;

(2) fusing the lymphocytes with myeloma cells (line X63Ag8.653) to form the hybridoma.

Patentansprüche für die Vertagsstaaten: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Monoklonaler Antikörper, welcher befähigt ist, mit einem zwischen zwei oder mehreren, genetisch nichtidentischen Individuen der menschlichen Bevölkerung geteilten Idiotypus auf einem humanen, anti-native-DNS-Antikörper zu reagieren.

2. Monoklonaler Antikörper des Anspruchs 1, welcher befähigt ist, mit einem Idiotyp auf humaner, anti-nativer DNS, welcher sich außerhalb der DNS-Bindungsstelle des anti-nativen DNS-Antikörper befindet, zu reagieren.

3. Monoklonaler Antikörper des Anspruchs 1 mit dem Ursprung Maus.

4. Monoklonaler Antikörper des Anspruchs 3, welcher von dem Hybridom sekretiert wird, das bei der American Type Culture Collection hinterlegt und als ATCC #HB8376 katalogisiert ist.

5. Monoklonaler Antikörper des Anspruchs 1, welcher nach dem folgenden Verfahren hergestellt worden ist:

(1) Erhalt von spezifisch gegen humane, anti-native-DNS-Antikörper sensibilisierten Lymphocyten;

(2) Verschmelzen der Lymphocyten mit Fusionspartnern unter Bildung von Hybridomen;

(3) Selektieren der Hybridome, welche einen Antikörper sekretieren, welcher befähigt ist, mit einer idiotypen Determinante auf einen humanen, anti-native-DNS-Antikörper aus wenigstens zwei genetisch nichtidentischen Individuen der menschlichen Bevölkerung zu reagieren;

(4) wahlweises Klonen der selektierten Hybridome; und

(5) Gewinnen d r monoklonal n Antikörper, welch befähigt sind, mit inem zwischen zwei oder m hreren, genetisch nichtidentischen Individuen der menschlichen Bevölkerung geteilten Idiotypus auf einem humanen, anti-native-DNS-Antikörper zu reagieren.

6. Monoklonaler Antikörper des Anspruchs 5, wobei Schritt (1) das Immunisieren eines Tieres mit einem humanen anti-DNS-Antikörper und das Isolieren von Milzzellen aus dem Tier umfaßt.

7. Monoklonaler Antikörper des Anspruchs 6, wobei das Tier eine Maus ist.

8. Monoklonaler Antikörper des Anspruchs 6, wobei der humane anti-native-DNS-Antikörper aus dem Zerfall eines Immunkomplexes erhalten wird, welcher DNS und einen humanen anti-DNS-Antikörper umfaßt, erhalten wird.

9. Monoklonaler Antikörper des Anspruchs 8, wobei der humane anti-DNS-Antikörper aus einem Patienten mit systemischem Lupus erythematosus stammt.

10. Monoklonaler Antikörper des Anspruchs 5, wobei der Fusionspartner eine Myelomzelle ist.

11. Monoklonaler Antikörper des Anspruchs 10, wobei die Myelomzelle X63Ag8.653 ist, welche bei der American Type Culture Collection hinterlegt und als CRL-1580 katalogisiert ist.

12. Verfahren zum Nachweis humaner anti-native-DNS-Antikörper, deren Anwesenheit in einer flüssigen Probe vermutet wird, umfassend das in-Kontakt-Bringen der Probe mit einem monoklonalen, anti-idiotypischen Antikörper des Anspruchs 1 und Bestimmen des Ausmaßes der Bindung.

13. Verfahren des Anspruchs 12, welches das

(i) Bilden eines Reaktionsgemisches durch in-Kontakt-Bringen der Probe mit einem markierten monoklonalen anti-idiotypen Antikörper des Anspruchs 1;

(ii) Ermöglichen, eine immunochemische Reaktion stattfinden zu lassen, wobei ein Teil des markierten Antikörpers einen Immunkomplex mit dem anti-native-DNS-Antikörper bildet und ein Teil frei bleibt;

(iii) Abtrennen des Immunkomplexes vom freien, markierten Antikörper;

(iv) Messen der Markierung in einer der Fraktionen; und

(v) das in-Bezug-Setzen der gemessenen Markierung zu der Menge an in der Probe ursprünglich befindlicher anti-nativer DNS umfaßt.

14. Verfahren des Anspruchs 13, wobei der Abtrennungsschritt (iii) die Zugabe eines Reagenzes, welches zur Präzipitierung des Immunkomplexes befähigt ist, zum Reaktionsgemisch umfaßt.

15. Verfahren des Anspruchs 14, wobei das Präzipitierungsreagenz ein heterologer Antikörper ist, welcher befähigt ist, mit dem anti-native-DNS-Antikörper zu reagieren.

16. Verfahren des Anspruchs 13, wobei die Markierung ein Radioisotop, ein Enzym, ein Chromophor oder Fluorophor ist.

17. Verfahren des Anspruchs 16, wobei das Radioisotop Iod-125 ist.

18. Verfahren des Anspruchs 16, wobei das Enzym Meerrettichperoxidase, α-Galactosidase oder alkalische Phosphatase ist.

19. Verfahren des Anspruchs 12, welches das

(i) in-Kontakt-Bringen der Probe mit einer festen Phase, auf welcher der vermutete anti-native-DNS-Antikörper adsorbiert ist;

(ii) in-Kontakt-Bringen der festen Phase mit einem monoklonalen, anti-idiotypen Antikörper des Anspruchs 1, wobei sich auf der festen Phase ein Immunkomplex bildet;

(iii) in-Kontakt-Bringen der festen Phase mit einem markierten Antikörper, welcher befähigt ist, mit dem monoklonalen Antikörper des Immunkomplexes zu reagieren;

(iv) Messen der auf der festen Phase adsorbierten markierten Menge; und

(v) das in-Bezug-Setzen der Menge an Markierung zu der Menge an in der Probe ursprünglich befindlichen anti-nativen DNS umfaßt.

20. Verfahren des Anspruchs 19, wobei das Näpfchen in einer Mikrotiterplatte die feste Phase ist.

21. Verfahren nach Anspruch 19, wobei der markierte Antikörper ein markierter Ratte-anti-(Maus-leichte Kette)-Antikörper ist.

22. Verfahren des Anspruchs 21, wobei die Markierung ein Radioisotop, ein Enzym, ein Chromophor oder Fluorophor ist.

23. Verfahren des Anspruchs 22, wobei die Markierung Schwefel-35 ist.

24. Verfahren des Anspruchs 22, wobei das Enzym Meerrettichperoxidase, α-Galactosidase oder alkalische Phosphatase ist.

25. Kit zur Bestimmung der Menge an humanem anti-native-DNS-Antikörper, dessen Anwesenheit in der flüssigen Probe vermutet wird, umfassend

(i) anti-native-DNS-Antikörper-positiv- und -negative-Kontrollen;

(ii) markierter, monoklonaler anti-idiotyper Antikörper des Anspruchs 1;

(iii) Reagenz, welches zur Präzipitation eines Immunkomplexes aus dem anti-native-DNS-Antikörper und dem markierten monoklonalen, anti-idiotypen Antikörper befähigt ist; und

(iv) Gebrauchsanweisungen für den Kit.

26. Kit des Anspruchs 25, worin die Markierung ein Radioisotop, ein Enzym, ein Chromophor oder Fluorophor ist.

27. Kit des Anspruchs 26, worin das Radioisotop Iod-125 ist.

28. Kit des Anspruchs 26, worin das Enzym Meerrettichperoxidase, α-Galactosidase oder alkalische Phosphatase ist.

29. Kit des Anspruchs 25, worin das Reagenz ein heterologer Antikörper ist, welcher befähigt ist, mit dem anti-native-DNS-Antikörper zu reagieren.

30. Hybridom, welches bei der American Type Culture Collection hinterlegt und als ATCC #HB8376 und dessen Mutationen katalogisiert ist.

Patentansprüche für den Vertragsstaat: AT

1. Verfahren zur Herstellung eines monoklonalen Antikörpers welcher befähigt ist, mit einem zwischen zwei oder mehreren, genetisch nichtidentischen Individuen der menschlichen Bevölkerung geteilten Idiotypus auf einem humanen anti-nativ -DNS-Antikörper zu reagieren, umfassend:

(1) Erhalt von spezifisch gegen humane anti-native-DNS-Antikörper sensibilisierten Lymphocyten;

(2) Verschmelzen der Lymphocyten mit Fusionspartnern unter Bildung von Hybridomen;

(3) Selektieren der Hybridome, welche einen Antikörper sekretieren, welcher befähigt ist, mit einer idiotypischen Determinante auf einem humanen, anti-nativen DNS-Antikörper aus wenigstens zwei genetisch nichtidentischen Individuen der menschlichen Bevölkerung zu reagieren;

(4) wahlweises Klonen der selektierten Hybridome; und

(5) Gewinnen der monoklonalen Antikörper.

2. Verfahren des Anspruchs 1, worin Schritt (1) das Immunosieren eines Tieres mit einem humanen anti-DNS-Antikörper und das Isolieren von Milzzellen aus dem Tier umfaßt.

3. Verfharen des Anspruchs 2, worin das Tier eine Maus ist.

4. Verfahren des Anspruchs 2, worin der humane anti-native-DNS-Antikörper aus dem Zerfall eines Immunkomplexes erhalten wird, welcher DNS und einen humanen anti-DNS-Antikörper umfaßt.

5. Verfahren des Anspruchs 4, worin der humane anti-DNS-Antikörper aus einem Patienten mit systemischem Lupus erythematosus stammt.

6. Verfahren des Anspruchs 1, worin der Fusionspartner eine Myelomzelle ist.

7. Verfahren des Anspruchs 6, worin die Myelomzelle X63Ag8.653 ist, welche bei der American Type Culture Collection hinterlegt und als CRL-1580 katalogisiert ist.

8. Verfahren zum Nachweis humaner anti-native-DNS-Antikörper, deren Anwesenheit in einer flüssigen Probe vermutet wird, umfassend das in-Kontakt-Bringen der Probe mit einem monoklonalen, anti-idiotypischen Antikörper des Anspruchs 1 und Bestimmen des Ausmaßes der Bindung.

9. Verfahren des Anspruchs 8, welches das

(i) Bilden eines Reaktionsgemisches durch in-Kontakt-Bringen der probe mit einem nach Anspruch 1 hergestellten markierten monoklonalen anti-idiotypen Antikörper;

(ii) Ermöglichen, eine immunochemische Reaktion stattfinden zu lassen, wobei ein Teil des markierten Antikörpers einen Immunkomplex mit dem anti-native-DNS-Antikörper bildet und ein Teil frei bleibt;

(iii) Abtrennen des Immunkomplexes vom freien, markierten Antikörper;

(iv) Messen der Markierung in einer der Fraktionen; und

(v) das in-Bezug-Setzen der gemessenen Markierung zu der Menge an in der Probe ursprünglich befindlichen anti-nativen DNS umfaßt.

10. Verfahren des Anspruchs 9, worin der Abtrennungsschritt (iii) die Zugabe eines Reagenzes, welches zur Präzipitierung des Immunkomplexes befähigt ist, zum Reaktionsgemisch umfaßt.

11. Verfahren des Anspruchs 10, worin das Präzipitierungsreagenz ein heterologer Antikörper ist, welcher befähigt ist, mit dem anti-native-DNS-Antikörper zu reagieren.

12. Verfahren des Anspruchs 9, worin die Markierung ein Radioisotop, ein Enzym, ein Chromophor oder Fluorophor ist.

13. Verfahren des Anspruchs 12, worin das Radioisotop Iod-125 ist.

14. Verfahren des Anspruchs 12, worin das Enzym Meerrettichperoxidase, α-Galactosidase oder alkalische Phosphatase ist.

15. Verfahren des Anspruchs 8, welches das

(i) in-Kontakt-Bringen der Probe mit einer festen Phase, auf welcher der vermutete anti-native-DNS-Antikörper adsorbiert ist;

(ii) in-Kontakt-Bringen der festen Phase mit einem monoklonalen, anti-idiotypen, nach Anspruch 1 hergestellten Antikörper, wobei sich auf der festen Phase ein Immunkomplex bildet;

(iii) in-Kontakt-Bringen der festen Phase mit einem markierten Antikörper, welcher befähigt ist, mit dem monoklonalen Antikörper des Immunkomplexes zu reagieren;

(iv) Messen der auf der festen Phase adsorbierten markierten Menge; und

(v) das in-Bezug-Setzen der Menge an Markierung zu der Menge an in der Probe ursprünglich befindlicher anti-nativer DNS umfaßt.

16. Verfahren des Anspruchs 15, worin das Näpfchen in einer Mikrotiterplatte die feste Phase ist.

17. Verfahren nach Anspruch 15, worin der markierte Antikörper ein markierter Ratte-anti-(Maus-leichte Kette)-Antikörper ist.

18. Verfahren des Anspruchs 17, worin die Markierung ein Radioisotop, ein Enzym, ein Chromophor oder Fluorophor ist.

19. Verfahren des Anspruchs 18, worin die Markierung Schwefel-35 ist.

20. Verfahren des Anspruchs 18, worin das Enzym Meerrettichperoxidase, α-Galactosidase oder alkalische Phosphatase ist.

21. Kit zur Bestimmung der Menge an humanem anti-native-DNS-Antikörper, dessen Anwesenheit in der flüssigen Probe vermutet wird, umfassend

(i) anti-native-DNS-Antikörper-positiv- und -negativ-Kontrollen;

(ii) markierter, monoklonaler anti-idiotyper, nach Anspruch 1 hergestellter Antikörper;

(iii) Reagenz, welches zur Präzipitation eines Immunkomplexes aus dem anti-native-DNS-Antikörper und dem markierten monoklonalen, anti-idiotypen Antikörper befähigt ist; und

(iv) Gebrauchsanweisungen für den Kit.

22. Kit des Anspruchs 21, worin die Markierung ein Radioisotop, ein Enzym, ein Chromophor oder Fluorophor ist.

23. Kit des Anspruchs 22, worin das Radioisotop Iod-125 ist.

24. Kit des Anspruchs 22, worin das Enzym Meerrettichperoxidase, α-Galactosidase oder alkalische Phosphatase ist.

25. Kit des Anspruchs 21, worin das Reagenz ein heterologer Antikörper ist, welcher befähigt ist, mit dem anti-native-DNS-Antikörper zu reagieren.

26. Verfahren zur Herstellung des Hybridoms, welches bei der American Type Culture Collection hinterlegt, und als ATCC #HB8376 und dessen Mutationen katalogisiert ist, umfassend

(1) die Gewinnung gegen anti-native-DNS-Antikörper spezifisch sensibilisierter Lymphocyten;

(2) das Verschmelzen der Lymphocyten mit Myelomzellen (Linie X63Ag8.653) unter Bildung des Hybridoms.

Revendications pour les Etats Contractants: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Un anticorps monoclonal capable de réagir avec un idiotype présent sur un anticorps humain anti-ADN natif et commun à deux ou plusieurs individus génétiquement non identiques de la population humaine.

2. L'anticorps monoclonal de la revendication 1, qui est capable de réagir avec un idiotype présent sur un anticorps humain anti-ADN natif qui est situé en dehors du site de liaison d'ADN de l'anticorps anti-ADN natif.

3. L'anticorps monoclonal de la revendication 1, qui est originaire de souris.

4. L'anticorps monoclonal de la revendication 3, qui est sécrété par l'hybridome déposé à American Type Culture Collection et inscrit sous la référence ATCC No. HB8376.

5. L'anticorps monoclonal de la revendication 1, qui a été produit par le procédé suivant:

(1) obtenir des lymphocytes spécifiquement sensibilisés à un anticorps humain anti-ADN natif;

(2) fusionner les lymphocytes avec des partenaires de fusion pour former des hybridomes;

(3) sélectionner les hybridomes qui sécrètent un anticorps capable de réagir avec un déterminant idiotypique présent sur un anticorps humain anti-ADN natif et provenant d'au moins deux individus génétiquement non identiques de la population humaine;

(4) facultativement, cloner les hybridomes sélectionnés; et

(5) recueillir les anticorps monoclonaux capables de réagir avec un idiotype présent sur un anticorps humain anti-ADN natif et commun à deux ou plusieurs individus génétiquement non identiques de la population humaine.

6. L'anticorps monoclonal de la revendication 5, dans lequel l'étape (1) consiste à immuniser un animal avec un anticorps humain anti-ADN et à isoler des cellules de rate de l'animal.

7. L'anticorps monoclonal de la revendication 6, dans lequel l'animal est une souris.

8. L'anticorps monoclonal de la revendication 6, dans lequel l'anticorps humain anti-ADN natif est obtenu par la dissociation d'un complexe immun comprenant de l'ADN et un anticorps humain anti-ADN.

9. L'anticorps monoclonal de la revendication 8, dans lequel l'anticorps humain anti-ADN provient d'un malade atteint de lupus érythémateux généralisé.

10. L'anticorps monoclonal de la revendication 5, dans lequel le partenaire de fusion est une cellule de myélome.

11. L'anticorps monoclonal de la revendication 10, dans lequel la cellule de myélome est X63Ag8.653 qui est déposé à American Type Culture Collection et inscrit sous la référence CRL-1580.

12. Une méthode pour détecter un anticorps humain anti-ADN natif dont la présence est soupçonnée dans un échantillon liquide, consistant à mettre l'échantillon en contact avec un anticorps anti-idiotypique monoclonal de la revendication 1 et à déterminer le degré de liaison.

13. La méthode de la revendication 12, qui consiste à:

(i) former un mélange réactionnel en mettant l'échantillon en contact avec un anticorps anti-idiotypique monoclonal de la revendication 1, marqué;

(ii) laisser s'effectuer une réaction immunochimique par laquelle une fraction de l'anticorps marqué forme un complexe immun avec l'anticorps anti-ADN natif et une fraction reste libre;

(iii) séparer le complexe immun de l'anticorps marqué libre;

(iv) mesurer la quantité de marqueur dans l'une des fractions; et

(v) rapporter la quantité mesurée du marqueur à la quantité d'anticorps humain anti-ADN natif initialement présente dans l'échantillon.

14. La méthode de la revendication 13, dans laquelle l'étape de séparation (iii) comprend l'addition au mélange réactionnel d'un réactif capable de précipiter le complexe immun.

15. La méthode de la revendication 14, dans laquelle le réactif précipitant est un anticorps hétérologue capable de réagir avec l'anticorps anti-ADN natif.

16. La méthode de la revendication 13, dans laquelle le marqueur est un radio-isotope, un enzyme, un chromophore ou un flu rophore.

17. La méthode de la revendication 16, dans laquelle le radio-isotope est l'iode-125.

18. La méthode de la revendication 16, dans laquelle l'enzyme est la peroxydase de raifort l'α-galactosidase ou la phosphatase alcaline.

19. La méthode de la revendication 12, qui consiste à:

(i) mettre l'échantillon en contact avec une phase solide sur laquelle est adsorbé l'anticorps anti-ADN natif soupçonné;

(ii) mettre la phase solide en contact avec un anticorps anti-idiotypique monoclonal de la revendication 1, si bien qu'un complexe immun se forme sur la phase solide;

(iii) mettre la phase solide en contact avec un anticorps marqué capable de réagir avec l'anticorps monoclonal du complexe immun;

(iv) mesurer la quantité de marqueur adsorbée sur la phase solide; et

(v) rapporter la quantité de marqueur à la quantité d'anticorps anti-ADN natif initialement présente dans l'échantillon.

20. La méthode de la revendication 19, dans laquelle la phase solide est le puits d'une plaque de microtitrage.

21. La méthode de la revendication 19, dans laquelle l'anticorps marqué est un anticorps de rat anti-(chaîne légère de souris) marqué.

22. La méthode de la revendication 21, dans laquelle le marqueur est un radio-isotope, un enzyme, un chromophore ou un fluorophore.

23. La méthode de la revendication 22, dans laquelle le marqueur est le soufre-35.

24. La méthode de la revendication 22, dans laquelle l'enzyme est la peroxydase de raifort, l'α-galactosidase ou la phosphatase alcaline.

25. Un kit pour déterminer la quantité d'anticorps humain anti-ADN natif dont la présence est soupçonnée dans un échantillon liquide, comprenant:

(i) des témoins positif et négatif d'anticorps anti-ADN natif;

(ii) un anticorps anti-idiotypique monoclonal de la revendication 1, marqué;

(iii) un réactif capable de précipiter le complexe immun de l'anticorps anti-ADN natif et de l'anticorps anti-idiotypique monoclonal marqué; et

(iv) des instructions pour l'emploi du kit.

26. Le kit de la revendication 25, dans lequel le marqueur est un radio-isotope, un enzyme, un chromophore ou un fluorophore.

27. Le kit de la revendication 26, dans lequel le radio-isotope est l'iode-125.

28. Le kit de la revendication 26, dans lequel l'enzyme est la peroxydase de raifort, l'α-galactosidase ou la phosphatase alcaline.

29. Le kit de la revendication 25, dans lequel le réactif est un anticorps hétérologue capable de réagir avec l'anticorps anti-ADN natif.

30. L'hybridome qui est en dépôt à American Type Culture Collection et inscrit sous la référence ATCC No. HB8376, et ses mutants.

Revendications pour l'Etat Contractant: AT

1. Procédé pour la production d'un anticorps monoclonal capable de réagir avec un idiotype présent sur un anticorps humain anti-ADN natif et commun à deux ou plusieurs individus génétiquement différents de la population humaine, consistant à:

(1) obtenir des lymphocytes spécifiquement sensibilisés à un anticorps humain anti-ADN natif;

(2) fusionner les lymphocytes avec des partenaires de fusion pour former des hybridomes;

(3) sélectionner les hybridomes qui sécrètent un anticorps capable de réagir avec un déterminant idiotypique présent sur un anticorps humain anti-ADN natif et provenant d'au moins deux individus génétiquement non identiques de la population humaine;

(4) facultativement, cloner les hybridomes sélectionnés; et

(5) recueillir les anticorps monoclonaux capables de réagir avec un idiotype présent sur un anticorps humain anti-ADN natif et commun à deux ou plusieurs individus génétiquement non identiques de la population humaine.

2. Procédé de la revendication 1, dans lequel l'étape (1) consiste à immuniser un animal avec un anticorps humain anti-ADN et à isoler des cellules de rate de l'animal.

3. Procédé de la revendication 2, dans lequel l'animal est une souris.

4. Procéd´ d la revendication 2, dans lequ l'anticorps humain anti-ADN natif est obt nu par la dissociati n d'un compl xe immun comprenant de l'ADN et un anticorps humain anti-ADN.

5. Procédé de la revendication 4, dans lequel l'anticorps humain anti-ADN provient d'un malade att int de lupus érythémateux g´néralisé.

6. Procédé de la revendication 1, dans lequ l l part nair de fusion est une cellule de myélome.

7. Procédé de la rev ndication 6, dans lequel la cellule de myélome est X63Ag8.653 qui est déposé à American Type Culture Collection t inscrit sous la référence CRL-1580.

8. Une méthode pour détecter un anticorps humain anti-ADN natif dont la présence est soupçonnée dans un échantillon liquide, consistant à mettre l'échantillon en contact avec un anticorps anti-idiotypique monoclonal de la revendication 1 et à déterminer l degré de liais n.

9. La méthod de la revendication 8, qui c nsiste à:

(i) former un mélange réactionnel en mettant l'échantillon en contact avec un anticorps anti-idiotypique monoclonal préparé selon la revendication 1, marqué:

(ii) laisser s'effectuer une réaction immunochimique par laquelle une fraction de l'anticorps marqué forme un complexe immun avec l'anticorps anti-ADN natif et une fraction reste libre;

(iii) séparer le complexe immun de l'anticorps marqué libre;

(iv) mesurer la quantité de marqueur dans l'une des fractions; et

(v) rapporter la quantité mesurée du marqueur à la quantité d'anticorps humain anti-ADN natif initialement présente dans l'échantillon.

10. La méthode de la revendication 9, da laquelle l'étape de séparation (iii) comprend l'addition au mélange réactionnel d'un réactif capable de précipiter le complexe immun.

11. La méthode de la revendication 10, dans laquelle le réactif précipitant est un anticorps hétérologue capable de réagir avec l'anticorps anti-ADN natif.

12. La méthode de la revendication 9, dans laquelle le marqueur est un radio-isotope, un enzyme, un chromophore ou un fluorophore.

13. La méthode de la revendication 12, dans laquelle le radio-isotope est l'iode-125.

14. La méthode de la revendication 12, dans laquelle l'enzyme est la peroxydase de raifort, l'α-galactosidase ou la phosphatase alcaline.

15. La méthode de la revendication 8, qui consiste à:

(i) mettre l'échantillon en contact avec une phase solide sur laquelle est adsorbé l'anticorps anti-ADN natif soupçonne;

(ii) mettre la phase solide en contact avec un anticorps anti-idiotypique monoclonal préparé selon la revendication 1, si bien qu'un complexe immun se forme sur la phase solide;

(iii) mettre la phase solide en contact avec un anticorps marqué capable de réagir avec l'anticorps monoclonal du complexe immun;

(iv) mesurer la quantité de marqueur adsorbée sur la phase solide; et

(v) rapporter la quantité de marqueur à la quantité d'anticorps anti-ADN natif initialement présente dans l'échantillon.

16. La méthode de la revendication 15, dans laquelle la phase solide est le puits d'une plaque de microtitrage.

17. La méthode de la revendication 15, dans laquelle l'anticorps marqué est un anticorps de rat anti-(chaîne légère de souris) marqué.

18. La méthode de la revendication 17, dans laquelle le marqueur est un radio-isotope, un enzyme, un chromophore ou un fluorophore.

19. La méthode de la revendication 18, dans laquelle le marqueur est le soufre-35.

20. La méthode de la revendication 18, dans laquelle l'enzyme est la peroxydase de raifort, l'α-galactosidase ou la phosphatase alcaline.

21. Un kit pour déterminer la quantité d'anticorps humain anti-ADN natif dont la présence est soupçonnée dans un échantillon liquide, comprenant:

(i) des témoins positif et négatif d'anticorps anti-ADN natif;

(ii) un anticorps anti-idiotypique monoclonal préparé selon la revendication 1, marqué;

(iii) un réactif capable de précipiter le complexe immun de l'anticorps anti-ADN natif et de l'anticorps anti-idiotypique monoclonal marqué; et

(iv) des instructions pour l'emploi du kit.

22. Le kit de la revendication 21, dans lequel le marqueur est un radio-isotope, un enzyme, un chromophore ou un fluorophore.

23. Le kit de la revendication 22, dans lequel le radio-isotope est l'iode-125.

24. Le kit de la revendication 22, dans lequel l'enzyme est la peroxydase de raifort, l'α-galactosidase ou la phosphatase alcaline.

25. Le kit de la revendication 21, dans lequel le réactif est un anticorps hétérologue capable de réagir avec l'anticorps anti-ADN natif.

26. Procédé pour la production de l'hybridome qui est en dépôt à American Type Culture Collection et inscrit sous la référence ATCC No. HB8376, et de ses mutants, consistant à:

(1) obtenir des lymphocytes spécifiquement sensibilisés à un anticorps humain anti-ADN natif;

(2) fusionner les lymphocytes avec des cellules de myélome (lignée X63Ag8.653) pour former l'hybridome.